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**PCT**  
**INTERNATIONAL PRELIMINARY EXAMINATION REPORT PCT**

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(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 91641	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. PCT/AU99/00306	International filing date ( <i>day/month/year</i> ) 23 April 1999	Priority Date ( <i>day/month/year</i> ) 23 April 1998
International Patent Classification (IPC) or national classification and IPC <b>Int. Cl.</b> <sup>7</sup> C12Q 1/68, 1/48; C12N 15/54		
<p>Applicant  <b>COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al</b></p>		

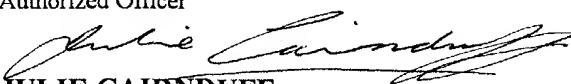
1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 52 sheet(s).

3. This report contains indications relating to the following items:

- I       Basis of the report
- II      Priority
- III     Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV     Lack of unity of invention
- V      Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI     Certain documents cited
- VII    Certain defects in the international application
- VIII    Certain observations on the international application

Date of submission of the demand 11 November 1999	Date of completion of the report 4 July 2000
Home and mailing address of the IPEA/AU  AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer   <b>JULIE CAIRNDUFF</b> Telephone No. (02) 6283 2545

**I. Basis of the report**

1. With regard to the **elements** of the international application:\*
- the international application as originally filed.
- the description, pages **1 to 4, 12 to 44**, as originally filed,  
pages , filed with the demand,  
pages **5 to 11**, received on **26 June 2000** with the letter of **26 June 2000**
- the claims, pages , as originally filed,  
pages , as amended (together with any statement) under Article 19,  
pages , filed with the demand,  
pages **62 to 72**, received on **26 June 2000** with the letter of **26 June 2000**
- the drawings, pages , as originally filed,  
pages , filed with the demand,  
pages **1/17 to 17/17**, received on **30 June 1999** with the letter of **30 June 1999**
- the sequence listing part of the description:  
pages , as originally filed  
pages , filed with the demand  
pages **1/17 to 17/17**, received on **30 June 1999** with the letter of **30 June 1999**
2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.  
These elements were available or furnished to this Authority in the following language which is:
- the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).  
 the language of publication of the international application (under Rule 48.3(b)).  
 the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:
- contained in the international application in written form.  
 filed together with the international application in computer readable form.  
 furnished subsequently to this Authority in written form.  
 furnished subsequently to this Authority in computer readable form.  
 The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
 The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
4.  The amendments have resulted in the cancellation of:
- the description, pages **45-61**  
 the claims, Nos.  
 the drawings, sheets/fig.
5.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Claims 1-50	YES
	Claims	NO
Inventive step (IS)	Claims 1-50	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-50	YES
	Claims	NO

**2. Citations and explanations (Rule 70.7)**

Citations

D1: AU 31341/95 (THE JOHN HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 1 February 1991

D2: Lee, W.H. et al. (1997) Cancer Epidemiology, Biomarkers and Prevention, volume 6, pp. 443-450

D3: Jhaveri, M.S. et al. (27 March 1998) Gene, volume 210, number 1, pp. 1-7

D4: Lee W.H. et al. (1994) Proceedings of the National Academy of Sciences USA, volume 91, pp. 11733-11737

D5: Frommer M. et al. (1992) Proceedings of the National Academy of Sciences USA, volume 89, number 5, pp. 1827-1831

Novelty and Inventive Step

Claims 1 to 50 are considered novel and inventive when compared to any one of D1 to D5. None of the citations disclose a method to identify abnormal methylation of cytosine within the glutathione-S-transferase (GST) Pi gene or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55, using a selective amplification technique. Furthermore none of D1 to D5 identify specific sequences that may be used in the exemplified method. As a result claims 1 to 50 are novel and inventive.

Industrial Applicability

Claims 1 to 46 exhibit industrial applicability.

said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) determining the presence of amplified DNA,

wherein the amplifying step (ii) is used to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.

Since the amplification is designed to only amplify the target region if the said site or sites at which abnormal cytosine methylation (i.e. as compared to the corresponding site or sites of DNA from subjects without the disease or condition being assayed) occurs is/are methylated, the presence of amplified DNA will be indicative of the disease or condition in the subject from which the isolated DNA has been obtained. The assay thereby provides a means for diagnosing or prognosing the disease or condition in a subject.

The step of isolating DNA may be conducted in accordance with standard protocols. The DNA may be isolated from any suitable body sample, such as cells from tissue (fresh or fixed samples), blood (including serum and plasma), semen, urine, lymph or bone marrow. For some types of body samples, particularly fluid samples such as blood, semen, urine and lymph, it may be preferred to firstly subject the sample to a process to enrich the concentration of a certain cell type (e.g. prostate cells). One suitable process for enrichment involves the separation of required cells through the use of cell-specific antibodies coupled to magnetic beads and a magnetic cell separation device.

Prior to the amplifying step, the isolated DNA is preferably treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine. This treatment permits the design of primers which enable the selective amplification of the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated.

Preferably, following treatment and amplification of the isolated DNA, a test is performed to verify that unmethylated cytosines have been efficiently converted to uracil or another nucleotide capable of forming a base pair with adenine, and that methylated cytosines have remained unchanged or efficiently converted to another nucleotide capable of forming a base pair with guanine.

Preferably, the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite in accordance with standard protocols. As will be clear from the above discussion of bisulphite treatment, unmethylated cytosines will be converted to uracil whereas methylated cytosines will be unchanged. Verification that unmethylated cytosines have been converted to uracil and that methylated cytosines have remained unchanged may be achieved by:

- (i) restricting an aliquot of the treated and amplified DNA with a suitable restriction enzyme(s) which recognise a restriction site(s) generated by or resistant to the bisulphite treatment, and
- (ii) assessing the restriction fragment pattern by electrophoresis.

Alternatively, verification may be achieved by differential hybridisation using specific oligonucleotides targeted to regions of the treated DNA where unmethylated cytosines would have been converted to uracil and methylated cytosines would have remained unchanged.

The amplifying step may involve polymerase chain reaction (PCR) amplification, ligase chain reaction amplification (20) and others (21).

Preferably, the amplifying step is conducted in accordance with standard protocols for PCR amplification, in which case, the reactants will typically be suitable primers, dNTPs and a thermostable DNA polymerase, and the conditions will be cycles of varying temperatures and durations to effect alternating denaturation of strand duplexes, annealing of primers (e.g. under high stringency conditions) and subsequent DNA synthesis.

To achieve selective PCR amplification with bisulphite-treated DNA, primers and conditions may be used to discriminate between a target region including a site or sites of abnormal cytosine methylation and a target region where there is no site or sites of abnormal cytosine methylation. Thus, for 5 amplification only of a target region where the said site or sites at which abnormal cytosine methylation occurs is/are methylated, the primers used to anneal to the bisulphite-treated DNA (i.e. reverse primers) will include a guanine nucleotide(s) at a site(s) at which it will form a base pair with a methylated cytosine(s). Such primers will form a mismatch if the target 10 region in the isolated DNA has unmethylated cytosine nucleotide(s) (which would have been converted to uracil by the bisulphite treatment) at the site or sites at which abnormal cytosine methylation occurs. The primers used for annealing to the opposite strand (i.e. the forward primers) will include a cytosine nucleotide(s) at any site(s) corresponding to site(s) of methylated 15 cytosine in the bisulphite-treated DNA.

Preferably, the primers used for the PCR amplification are of 12 to 30 nucleotides in length and are designed to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition 20 being assayed. In addition, the primers preferably include a terminal nucleotide that will form a base pair with a cytosine nucleotide (reverse primer), or the guanine nucleotide opposite (forward primer), that is abnormally methylated in the DNA of a subject with the disease or condition being assayed.

25 The step of amplifying is used to amplify a target region within the GST-Pi gene and/or its regulatory flanking sequences. The regulatory flanking sequences may be regarded as the flanking sequences 5' and 3' of the GST-Pi gene which include the elements that regulate, either alone or in combination with another like element, expression of the GST-Pi gene.

In particular, the step of amplifying is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 (wherein the numbering of the CpG sites is relative to the transcription start site). The numbering and position of CpG sites is shown in Figure 1.

The step of determining the presence of amplified DNA may be conducted in accordance with standard protocols. One convenient method involves visualisation of a band(s) corresponding to amplified DNA, following gel electrophoresis.

Preferably, the disease or condition to be assayed is selected from cancers, especially hormone dependent cancers such as prostate cancer, breast cancer and cervical cancer, and liver cancer.

For the diagnosis or prognosis of prostate cancer, the step of amplifying preferably amplifies a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53, more preferably, -43 to +10. However, within these target regions it is believed that there are CpG sites which show variability in methylation status in prostate cancer or are methylated in other tissues. Thus, for the target region defined by (and inclusive of) CpG sites -43 to +10, it is preferred that the primers used for amplification be designed so as to minimise (i.e. by use of redundant primers or by avoidance of the sites) the influence of CpG sites -36, -32, -23, -20, -19, -14 and a polymorphic region covering site -33. Further, for DNA isolated from cells other than from prostate tissue (e.g. blood), it is preferred that the primers used be designated to amplify a target region that does not include the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -7 to +7, or, more preferably, -13 to +8, since this may lead to false positives. Further preferred target regions, therefore, are within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14, -43 to -8, +9 to +53 and +1 to +53.

Suitable primer pairs for the diagnosis or prognosis of prostate cancer, include those consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers (i.e. anneal to the 5' end of the target region)

5	CGCGAGGTTTCGTTGGAGTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGACTACGCCCGTTC	(SEQ ID NO: 2)
	YGGTITTAGGAATTTTTTCGC	(SEQ ID NO: 3)
	YGGYGYGTTAGTTYGTTGYGTATATTTC	(SEQ ID NO: 4)
	GGGAATTTCGCGATGTTYGGCGC	(SEQ ID NO: 5)
10	TTTTTAGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTATCGC	(SEQ ID NO: 7)

Reverse Primers (i.e. anneal to the extension of the forward primer)

15	TCCCCTCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 8)
	GAAACGCTCCGAACCCCTAAAAACCGCTAACCG	(SEQ ID NO: 9)
	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 10)
	ACCCCRACRACCRCTACACCCRAACGTCG	(SEQ ID NO: 11)
	CTCTTCTAAAAATCCRCRAACTCCCAGCG	(SEQ ID NO: 12)
	AAAACRCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 13)
	AACTCCCRCGACCCAACCCGACGACCG	(SEQ ID NO: 14)
20	AAAAATTCTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 15)
	AAAAACCRAAATAAAACCAACACGACG	(SEQ ID NO: 16)

wherein Y is C, T or, preferably, a mixture thereof, and R is A, G or, preferably, a mixture thereof.

For the diagnosis or prognosis of liver cancer, the step of amplifying preferably amplifies a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14 and/or +9 to +53. However, within these target regions it is believed that there are CpG sites which show variability in methylation status in liver cancer or are methylated in other tissues. Thus, for the target region defined by (and inclusive of) CpG sites -43 to -14, it is preferred that the primers used

for amplification be designed so as to minimise (i.e. by use of redundant primers or by avoidance of the sites) the influence of CpG sites -36, -32, -23, -20, -19, -14 and a polymorphic region covering site -33.

It will be appreciated by persons skilled in the art, that a site or sites of abnormal cytosine methylation within the above identified target regions of the GST-Pi gene and/or its regulatory flanking sequences, could be detected for the purposes of diagnosing or prognosing a disease or condition (particularly, prostate cancer and/or liver cancer) by methods which do not involve selective amplification. For instance, oligonucleotide/polynucleotide probes could be designed for use in hybridisation studies (e.g. Southern blotting) with bisulphite-treated DNA which, under appropriate conditions of stringency, selectively hybridise only to DNA which includes a site or sites of abnormal methylation of cytosine(s). Alternatively, an appropriately selected informative restriction enzyme(s) could be used to produce restriction fragment patterns that distinguish between DNA which does and does not include a site or sites of abnormal methylation of cytosine(s).

Thus, in a second aspect, the present invention provides a diagnostic or prognostic assay for a disease or condition in a subject said disease or condition characterised by abnormal methylation of cytosine at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;

- (i) isolating DNA from said subject, and
- (ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.

The step of isolating DNA may be conducted as described above in relation to the assay of the first aspect.

Preferably, the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is selected from the regions defined by (and inclusive of)

CpG sites -43 to +53, -43 to +10, -43 to -14, +9 to +53 and +1 to +53.

However, within these regions, it is preferred that certain sites (namely, CpG sites, -36, -33, -32, -23, -20, -19, and -14) be avoided as the site or sites at which, for the purpose of the assay, the presence of abnormal methylation of cytosine is determined.

Where the determination step is to involve selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probes, prior to the determination step, the isolated DNA is preferably treated (e.g. with bisulphite) such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine. This treatment permits the design of probes which allow for selective hybridisation to a target region including a site or sites of abnormal methylation of cytosine.

In a third aspect, the present invention provides a primer or probe (sequence shown in the 5' to 3' direction) comprising a nucleotide sequence selected from the group consisting of:

	CGCGAGGTTTCGTTGGAGTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGAGTACGCGCGGTTTC	(SEQ ID NO: 2)
20	YGGTTTTAGGGAATTTTTTCGCG	(SEQ ID NO: 3)
	YGGYGYGTTAGTTYGTTGYGTATATTTC	(SEQ ID NO: 4)
	GGGAATTTTTTCGCGATGTTYGCGC	(SEQ ID NO: 5)
	TTTTTAGGGGGTTYGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTATCGC	(SEQ ID NO: 7)
25	AAAAATTCTRAATCTCTCCGAATAAACG	(SEQ ID NO: 8)
	AAAAACCRAAATAAAACCACACGACG	(SEQ ID NO: 9)
	TCCCCTCCCTCCCCGAAACCGCTCCG	(SEQ ID NO: 10)
	GAAACGCTCCGAACCCCTAAAAACCGCTAACCG	(SEQ ID NO: 11)
	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 12)
30	ACCCCRACRACRCTACACCCRAACGTCG	(SEQ ID NO: 13)

Claims:

1. A diagnostic or prognostic assay for a disease or condition in a subject,  
5 said disease or condition characterised by abnormal methylation of cytosine at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;
  - (i) isolating DNA from said subject,
  - (ii) exposing said isolated DNA to reactants and conditions for the  
10 amplification of a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the disease or condition occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are  
15 methylated, and
    - (iii) determining the presence of amplified DNA,  
wherein the amplifying step (ii) is used to amplify a target region within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.
- 20 2. An assay according to claim 1, wherein prior to the amplifying step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.  
25 3. An assay according to any one of the preceding claims, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.
- 30 4. An assay according to claim 3, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the

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reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).  
5

5. An assay according to claim 4, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject  
10 with the disease or condition being assayed.

6. An assay according to claim 5, wherein the primers are of 12 to 30 nucleotides in length.

15 7. An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.

20 8. An assay according to claim 2, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

9. An assay according to claim 8, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

25 10. An assay according to claim 9, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with  
30 uracil.

11. An assay according to claim 10, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.  
5
12. An assay according to claim 11, wherein the primers are of 12 to 30 nucleotides in length.
- 10 13. An assay according to claim 12, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.
- 15 14. An assay according to any one of the preceding claims, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.
- 20 15. An assay according to any one of the preceding claims, wherein the disease or condition to be assayed is selected from cancers.
16. An assay according to claim 15, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.  
25
17. An assay according to claim 16, wherein the disease or condition to be assayed is prostate cancer.
18. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its  
30

regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53.

19. An assay according to claim 17, wherein the amplifying step is used to  
5 amplify a target region within the region of the GST-Pi gene and its  
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to  
+10.

20. An assay according to claim 17, wherein the amplifying step is used to  
10 amplify a target region within the region of the GST-Pi gene and its  
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to  
-14.

21. An assay according to claim 17, wherein the amplifying step is used to  
15 amplify a target region within the region of the GST-Pi gene and its  
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to  
-8.

22. An assay according to any one of the preceding claims wherein the  
20 target region excludes any or all of the CpG sites -36, -32, -23, -20, -19 and  
-14.

23. An assay according to any one of claims 5 to 21, wherein if either or  
both of the reverse or forward primers anneal to a sequence within the target  
25 region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then  
said PCR amplification further utilises equivalent reverse and/or forward  
primers including a redundant nucleotide(s) at the position(s) within their  
sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -  
36, -32, -23, -20, -19 and -14.

24. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

5

25. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

10

26. An assay according to claim 17, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

15	CGCGAGGTTTCGTTGGAGTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGAGTACGCCGGTTC	(SEQ ID NO: 2)
	YGGTTTAGGAAATTTTTTCGC	(SEQ ID NO: 3)
	YGGYGYGTAGTTGTTGYGTATATTTC	(SEQ ID NO: 4)
	GGGAATTTCGCGATGTTYGCGC	(SEQ ID NO: 5)
20	TTTITAGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTATCGC	(SEQ ID NO: 7)

Reverse Primers

25	TCCCCTCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 8)
	GAAACGCTCCGAACCCCTAAAAACCGCTAACG	(SEQ ID NO: 9)
	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 10)
	ACCCCRACRACRCTACACCCRAACGTCG	(SEQ ID NO: 11)
	CTCTTCTAAAAATCCRCRAACTCCGCCG	(SEQ ID NO: 12)
	AAAACRCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 13)
	AACTCCCRCGACCCCAACCCGACGACCG	(SEQ ID NO: 14)
30	AAAAATTCTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),  
wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

27. An assay according to claim 17, wherein the amplifying step involves  
5 PCR amplification using primer pairs consisting of a forward and reverse  
primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTCGTTGGAGTTCGTCGTC (SEQ ID NO: 1)  
CGTTATTAGTGAGTACGCGCGGTTTC (SEQ ID NO: 2)

10 Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)  
GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

28. An assay according to claim 17, wherein the amplifying step involves  
15 PCR amplification using primer pairs consisting of a forward and reverse  
primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTCGC (SEQ ID NO: 3)  
YGGYGYGTTAGTTYGTTGYGTATATTC (SEQ ID NO: 4)  
20 GGGAAATTTTTTCGCGATGTTYGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)  
ACCCCRACRACRCTACACCCCRAACGTCG (SEQ ID NO: 11)  
CTCTTCTAAAAAATCCRCRAACTCCGCCG (SEQ ID NO: 12)  
25 AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)  
AACTCCCRCGACCCCAACCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

29. An assay according to claim 17, wherein the amplifying step involves  
30 PCR amplification using primer pairs consisting of a forward and reverse

primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTYYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTATCGC (SEQ ID NO: 7)

5 Reverse Primers

AAAAATTCAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

10 30. An assay according to claim 16, wherein the disease or condition to be assayed is liver cancer.

15 31. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

20 32. An assay according to claim 31, wherein the target region excludes any or all of the CpG sites -36, -32, -23, -20, -19, and -14.

25 33. An assay according to claim 30, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said PCR amplification further utilises equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites - 36, -32, -23, -20, -19 and -14.

30 34. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its

regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

35. A diagnostic or prognostic assay for a disease or condition in a subject  
5 said disease or condition characterised by abnormal methylation of cytosine  
at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its  
regulatory flanking sequences, wherein said assay comprises the steps of;  
(i) isolating DNA from said subject, and  
(ii) determining the presence of abnormal methylation of cytosine at a site  
10 or sites within the region of the GST-Pi gene and/or its regulatory flanking  
sequences defined by (and inclusive of) CpG sites -43 to +55.

36. An assay according to claim 35, wherein the region of the GST-Pi gene  
and its regulatory flanking sequences within which the presence of  
15 methylated cytosine(s) at a site or sites is determined is selected from the  
regions defined by (and inclusive of) CpG sites -43 to +53, -43 to +10, -43 to  
-14, +9 to +53 and +1 to +53.

37. An assay according to claim 35 or 36, wherein the said region of the  
20 GST-Pi gene and its regulatory flanking sequences excludes any or all of the  
CpG sites -36, -32, -23, -20, -19 and -14.

38. An assay according to claim 36, wherein the region of the GST-Pi gene  
and its regulatory flanking sequences within which the presence of  
25 methylated cytosine(s) at a site or sites is determined is the region defined by  
(and inclusive of) CpG sites +9 to +53.

39. An assay according to claim 36, wherein the region of the GST-Pi gene  
and its regulatory flanking sequences within which the presence of

methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +1 to +53.

40. An assay according to any one of claims 35 to 39, wherein prior to the 5 determination step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.
- 10 41. An assay according to claim 40, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.
- 15 42. An assay according to any one of claims 35 to 41, wherein the determination step involves selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probe(s).
- 20 43. An assay according to claim 42, wherein if the probe(s) hybridise to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said selective hybridisations further utilises equivalent probe(s) including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.
- 25 44. An assay according to any one of claims 35 to 43, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.
45. An assay according to any one of claims 35 to 43, wherein the disease or condition to be assayed is selected from cancers.

46. An assay according to claim 45, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

5 47. An assay according to claim 46, wherein the disease or condition to be assayed is prostate cancer.

48. An assay according to claim 46, wherein the disease or condition to be assayed is liver cancer.

10

49. A primer or probe comprising a nucleotide sequence selected from the group consisting of:

	CGCGAGGTTTCGTTGGAGTTCGTCGTC	(SEQ ID NO: 1)
	CGTTATTAGTGAGTACGCGCGGTTC	(SEQ ID NO: 2)
15	YGGTTTTAGGAAATTTTTTCGC	(SEQ ID NO: 3)
	YGGYGYGTTAGTTYGTTGYGTATATTC	(SEQ ID NO: 4)
	GGGAATTTTTTCGCGATGTTYGCGC	(SEQ ID NO: 5)
	TTTTTAGGGGTTYGAGCGTTTC	(SEQ ID NO: 6)
	GGTAGGTTGYGTTATCGC	(SEQ ID NO: 7)
20	AAAAATTCTRAATCTCTCCGAATAAACG	(SEQ ID NO: 8)
	AAAAACCRAAATAAAAACCACACGACG	(SEQ ID NO: 9)
	TCCCCTCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 10)
	GAAACGCTCCGAACCCCTAAAAACCGCTAACG	(SEQ ID NO: 11)
	CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 12)
25	ACCCCRACRACCRCTACACCCRAACGTG	(SEQ ID NO: 13)
	CTCTTCTAAAAATCCRCRAACTCCGCCG	(SEQ ID NO: 14)
	AAAACRCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 15)
	AACTCCCRCGACCCCAACCCCGACGACCG,	(SEQ ID NO: 16),

wherein Y is a mixture of C and T, and R is a mixture of A and G.

30

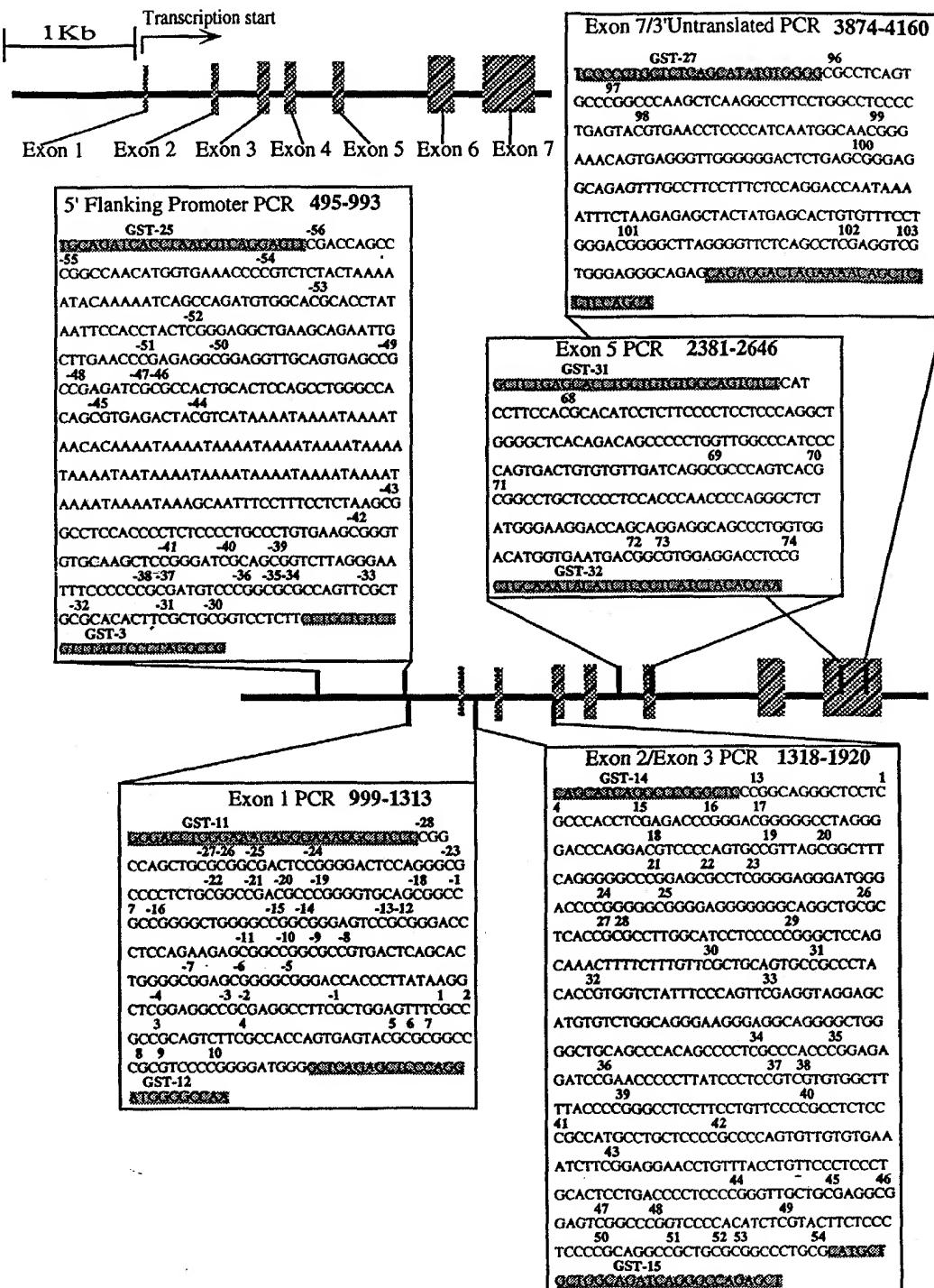
50. A probe comprising a nucleotide sequence selected from the group consisting of:

AAACCTAAAAATAAACAAACAA (SEQ ID NO: 17)  
GGGCCTAGGGAGTAAACAGACAG (SEQ ID NO: 18)  
5 CCTTTCCCTCTTCCCARRTCCCCA (SEQ ID NO: 19)  
TTTGGTATTTTTTCGGGTTTAG (SEQ ID NO: 20)  
CTTGGCATCCTCCCCGGGCTCCAG (SEQ ID NO: 21)  
GGYAGGGAAGGGAGGYAGGGGYTGGG (SEQ ID NO: 22).

10

FIGURE 1

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**Figure 2** Upstream Region of Differential Methylation in Prostate Cancer

<pre> ATAAAATAAA ATAAAGCAAT TTCCCTTCCT CTAAGCGGCC TCCACCCCTC TCCCCTGCC -42 ATAAAATAAA ATAAAGATAAT TTGTGGTTT TTAAGTGGTT TTATTTTTT TTTTTTGTGTT TGTGAAGTGGG -355 ATAAAATAAA ATAAAGATAAT TTGTGGTTT TTAAGCGGGT TTATTTTTT TTTTTTGTGTT TGTGAAGCGGG B-U </pre>	<pre> -41      -40      -39      -38-37    -36-35-34    -33      -32 GTGTGCAAGC TCCGGGATCG CAGGGTCTT AGGGAATTTC CCCCGCGAT GTCCCCGGCGC GCCAGTTCGC TGCAGCACAT -275 GTGTGTAAGT TTGGGATTG TAGTGGTTT AGGGAATTTC TTGGTGTGAT GTTTTGGTGT GTTAGTTGT GTGTATATT B-U GTGTGTAAGT TTGGGATCG TAGGGTTT AGGGAATTTC TTGGTGTGAT GTTTCGGCGC GTTAGTTCGT TGCATATT B-M </pre>	<pre> CGPS-5 YGGTTT AGGGAATTTC TTTCGC&gt;CGPS-6 YGGYGY GTTAGTTGT TGYGTATATT CGPS-11 GGAATTTC TTTCGCAT GTTTYGGCGC&gt; </pre>	<pre> -31      -30      -29      -28 TCGCTGGGT CCTCTTCCTG CTGCTGTG ACTCCCTAGG CCCCGCTGGG GACCTGGGAA AGAGGGAAAG GCTTCCCCGG -195 TTGTTGGT TTTTTTTTG TTGTTGTGTT ATTGTTAGG TTTGTTGGG GATTGGGAA AGAGGGAAAG GTTTTTTGG B-U TCGTTGGGT TTTTTTTTG TTGTTGTGTT ATTGTTAGG TTTGTTGGG GATTGGGAA AGAGGGAAAG GTTTTTTCGG B-M </pre>	<pre> TC&gt; </pre>	<pre> -27-26-25 -24      -23      -22      -21      -20      -19      -18      -17      -16 CCAGCTGGC GGGACTCCG GGGACTCCAG GGCGCCCTC TGCGGCCGAC GCCCCGGGTG CAGGGCCGC CGGGGCTGGG -115 TTAGTGTGT GGTGATTTC GGGATTTCAG GGTGTTTGT GGTGTTTGT TAGTGGTTGT GTTTGGTGGG B-U TTAGTGCAC GGGGATTTCG GGGGATTTCAG GGCCTTTT GTCGGTCGAC GTTCGGGTG TAGCGGTGCT CGGGGTTGGG B-M </pre>	<pre> &lt;GGC CCRCTAAARC CCCTAAATC CCRC CGPS-7 &lt; GCTG CAARCCCCAC ATCRCCARCA RCCCCA CGPS-8 &lt;G CGCTAAAGC CCCTAAATC CCRCAAAA CGPS-12 &lt;GCCARCA GCCCAAACCC </pre>
--	--	---	---	---------------------	---	---

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Figure 2 (Continued)

-15 -14 -13-12 -11 -10 -9 -8 -7 -6 -5  
GCCGGGGGA GTCCGGGGGA CCCTCCAGAA GAGGGCGGG CAGCACTGGG GCGGAGCGGG GCGGGACCCAC -35  
GTGGTGGGA GTTGTGGGA TTTTTAGAA GAGTTAGAA TAGTATGGG GTGGAGTGG GTGGATTAT B-U  
GTCGGGGGA GTTCGGGGAA TTTTTAGAA GAGGGCTGG CGTCGTGATT TAGTATGGG GCGGAGCGGG GCGGGATTAT B-M

<GCCGCCT CAARCRCCCT AAAAATCTT CTC CGPS-9  
CAGCCRCCTT CAA CGPS-13

-4 -3 -2 -1 > 1 2 3 4 5 6 7  
CCTTATAAGG CTCGGAGGCC GCGAGGCCCT CGCTGGAGT TCGCCGCCGC AGTCTTCGCC ACCAGTGAGT ACGCGCGGCC +46  
TTTATAAGG TTGGAGGT GTGAGGT TGTGGAGT TTGTTGTGT AGTTTTGTT ATTAGTGAGT ATGTTGGGT B-U  
TTTATAAGG TCAGGAGTC GCGAGGT TGTGGAGT CGTGGAGT AGTTTCGTT ATTAGTGAGT ACGCGCGGT B-M

CGPS-1 C GCGAGGT TGTGGAGT TCGTCGTC> CGPS-2 CGTT ATTAGTGAGT ACGCGCGGT

8 9 10  
CGCGTCCCCG GGGATGGGC TCAAGACTCC CAGCATGGG CCAA +90  
TGTGTTTTTG GGGATGGGT TTAGAGTT TAGTATGGG TTAA B-U  
CGCGTTTCG GGGATGGGT TTAGAGTT TAGTATGGG TTAA B-M

C>

**Figure 3A** Methylation Status of Individual Sites in the GST-Pi Gene

site	LN	Du	PC3 M	PC3 MM	2AN BN CN	2AC	BC	CC	DC	XC	WC	Pr
-28	+++	-	++	-	-	++	+	++	+++	++	-	-
-27	+++	-	++	++	-	+	+	++	+++	++	-	-
-26	+++	-	++	++	-	+	+	++	+++	++	-	-
-25	+++	-	++	++	-	+	++	++	+++	++	-	-
-24	+++	-	++	++	-	+	++	++	+++	++	-	-
-23	+++	-	+	+	-	++	++	++	++	-	+++	-
-22	+++	-	+	+	-	++	++	++	++	-	+++	-
-21	+++	-	+	-	-	++	++	++	++	-	+++	-
-20	+++	-	-	-	-	++	++	++	++	-	+	-
-19	+++	-	++	-	-	+	++	++	++	-	+	-
-18	+++	-	++	-	-	++	++	++	++	-	++	-
-17	+++	-	+	-	-	++	++	++	++	-	++	-
-16	+++	-	++	-	-	+	++	++	++	-	++	-
-15	+++	-	+	-	-	+	++	++	++	-	++	-
-14	+++	-	-	-	-	+	++	++	++	-	+	-
-13	+++	-	-	-	-	+	++	++	++	B	++	+
-12	+++	-	-	-	-	+	++	++	++	B	++	+
-11	+++	-	-	-	-	+	++	++	++	B	++	+
-10	+++	-	-	-	-	+	++	++	++	B	++	+
-9	+++	-	-	-	-	+	++	++	++	B	++	+
-8	+++	-	-	-	-	+	++	++	++	B	++	+
-7	+++	-	-	-	-	+	++	++	++	B	++	+
-6	+++	-	-	-	-	+	++	++	++	B	++	+
-5	+++	-	-	-	-	+	++	++	++	B	++	+
-4	+++	-	-	-	-	+	++	++	++	B	++	+
-3	+++	-	-	-	-	+	++	++	++	B	++	+
-2	+++	-	-	-	-	+	++	++	++	B	++	+
-1	+++	-	-	-	-	+	++	++	++	B	++	+

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Figure 3A (cont'd)

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Figure 3A (cont'd)



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**Figure 3B** Methylation Status of Individual Sites in the GST-Pi Gene

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FIGURE 3B (cont'd)

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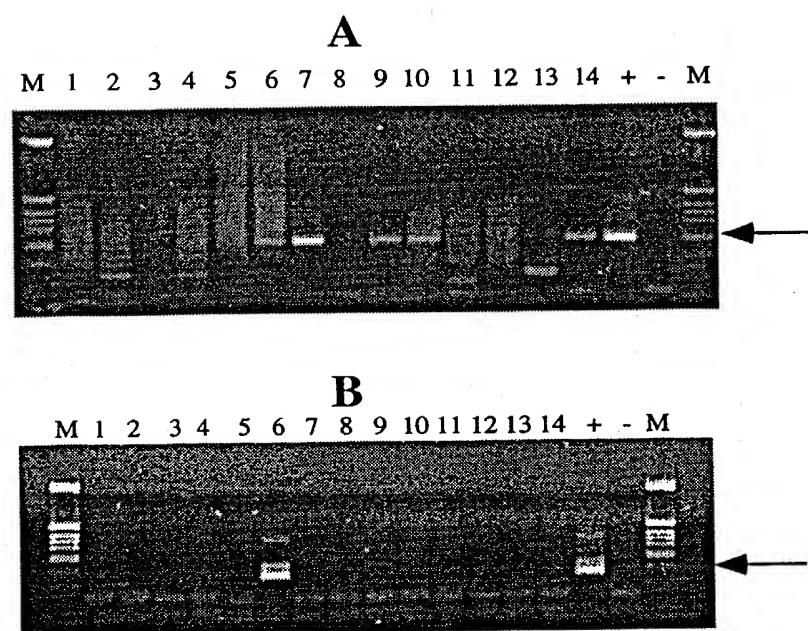
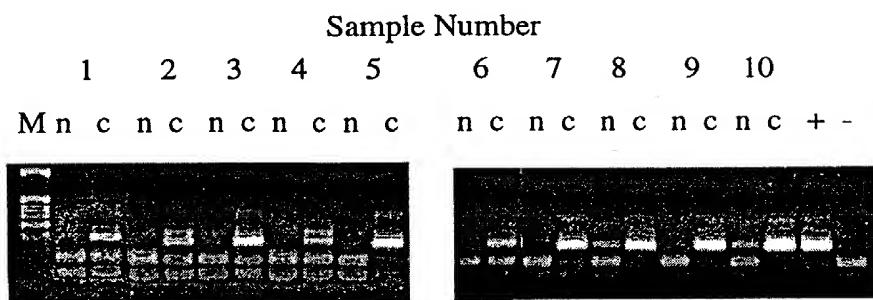


FIGURE 4A

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Sample	Tissue	Gleason	% Methylation	
			Non CG rich PCR	
1	Normal	N/A	-	
	Cancer	3+3	++++	
2	Normal	N/A	-	
	Cancer	3+5	++	
3	Normal	N/A	-	
	Cancer	3+3	++	
4	Normal	N/A	-	
	Cancer	3+5	-	
5	Normal	N/A	-	
	Cancer	2+2	++	
6	Normal	N/A	-	
	Cancer	3+3	-	
7	Normal	N/A	-	
	Cancer	2+3	++	
8	Normal	N/A	-	
	Cancer	3+3	++	
9	Normal	N/A	-	
	Cancer	2+3	++++	
10	Normal	N/A	-	
	Cancer	?	++	

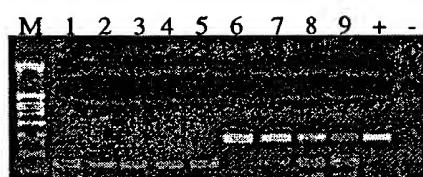
FIGURE 4B

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A



B



C

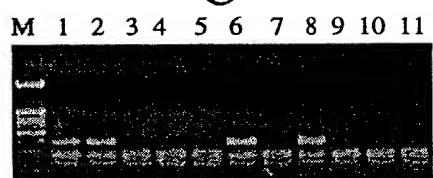
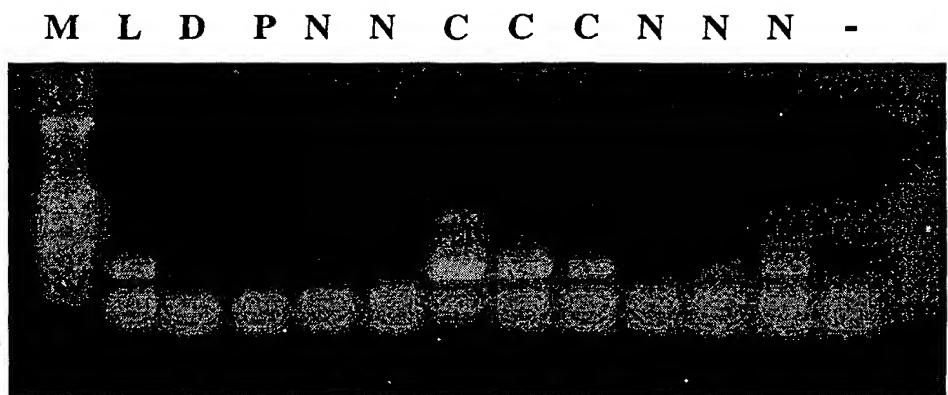


FIGURE 4C

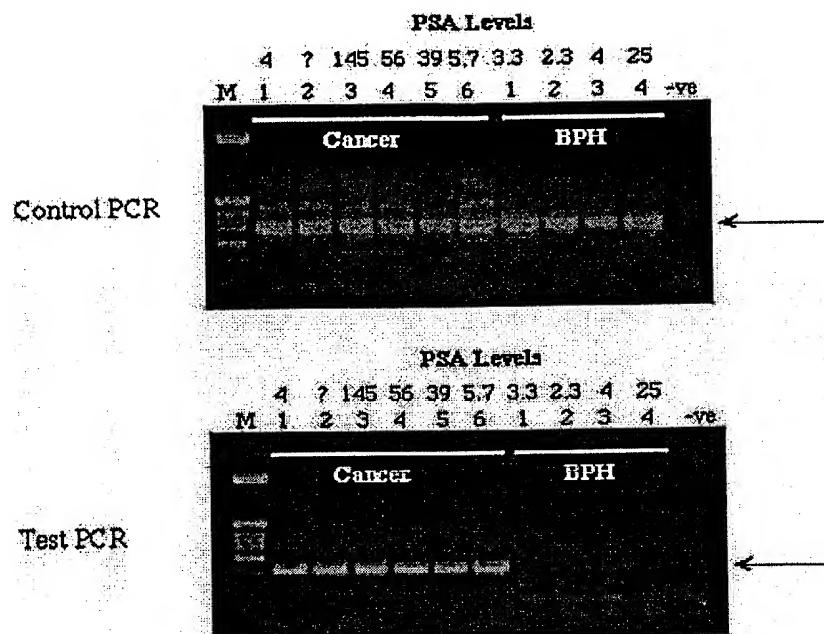
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**Figure 5**



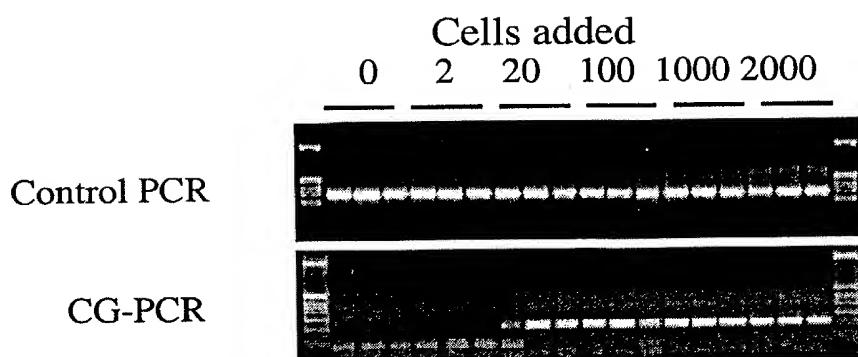
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**Figure 6**

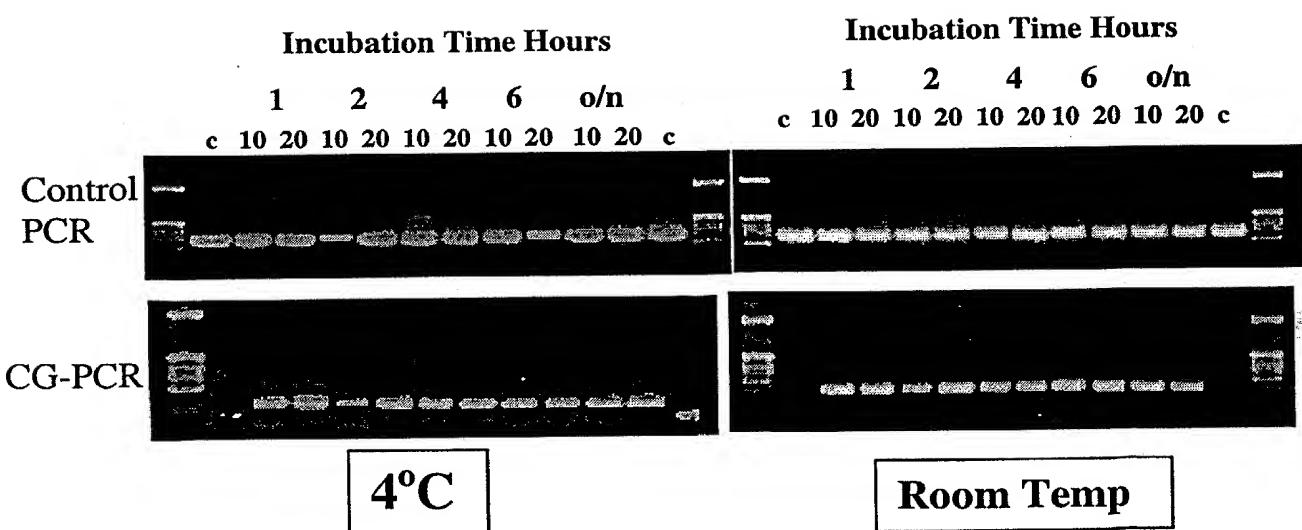


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**Figure 7A**

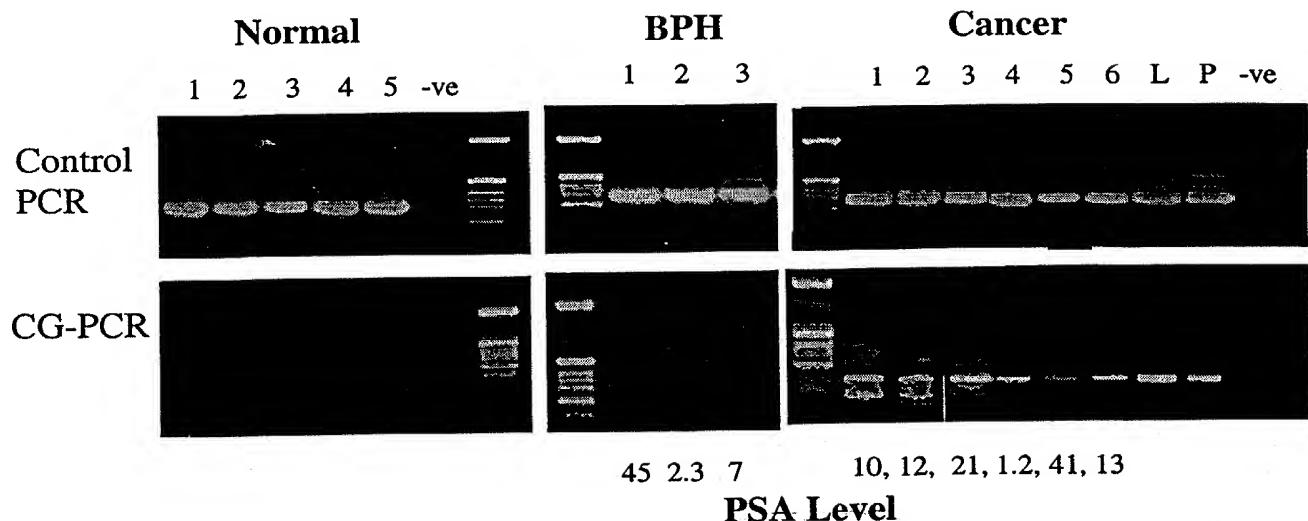


**Figure 7B**



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**Figure 8**



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**Figure 9**

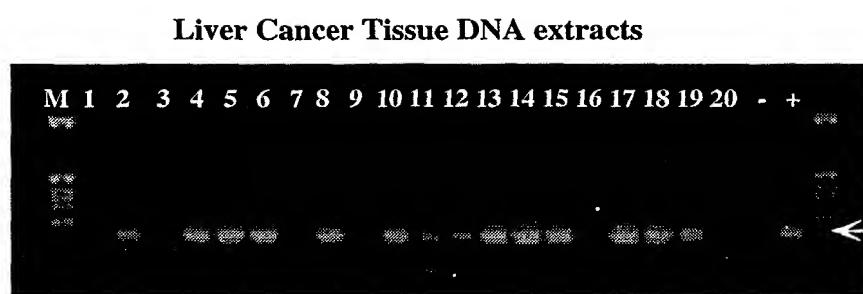
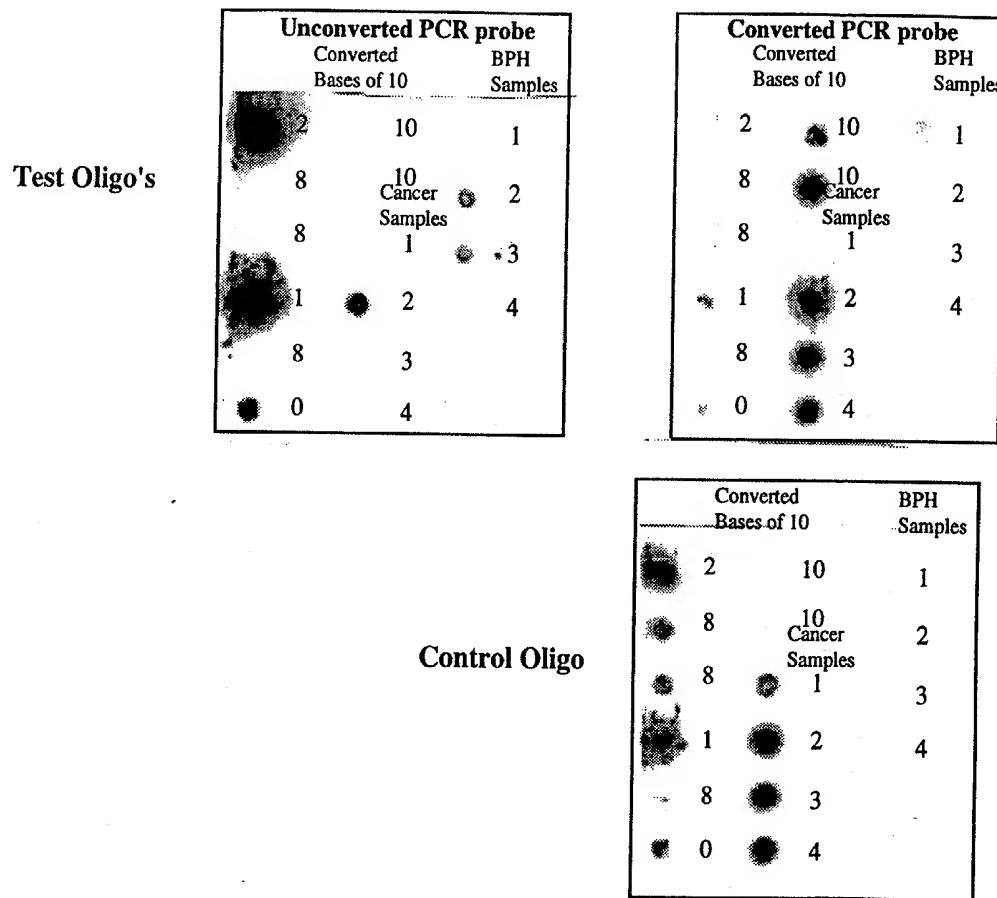


Figure 10



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**Sequence Listings:**

Applicant: Commonwealth Scientific and Industrial Research Organisation

Title: Diagnostic assay

Prior Application Number: PP3129

Prior Application Filing Date: 1998-04-23

Number of SEQ ID NOs: 59

Software: PatentIn Ver. 2.1

SEQ ID NO: 1

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 1

cgcgaggttt tcgttggagt ttcgtcgtc

29

SEQ ID NO: 2

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 2

cgttatttagt gagtacgcgc ggttc

25

SEQ ID NO: 3

Length: 24

Type: DNA

Organism: Homo sapiens

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Sequence: 3

ygggttttagg gaattttttt tcgc

24

SEQ ID NO: 4

Length: 28

Type: DNA

Organism: Homo sapiens

Sequence: 4

ygggygygtta gtttgttggyg tatatttc

28

SEQ ID NO: 5

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 5

ggaaatttttt ttccgcgatg tttyggcgc

29

SEQ ID NO: 6

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 6

tttttagggg gtttggagcg ttcc

24

SEQ ID NO: 7

Length: 19

Type: DNA

Organism: Homo sapiens

Sequence: 7

ggtaggttgy gtttatcgc

19

SEQ ID NO: 8

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Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 8

aaaaattcra atctctccga ataaacg

27

SEQ ID NO: 9

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 9

aaaaaccraa ataaaaacca cacgacg

27

SEQ ID NO: 10

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 10

tcccatccct ccccgaaacg ctccg

25

SEQ ID NO: 11

Length: 33

Type: DNA

Organism: Homo sapiens

Sequence: 11

gaaacgctcc gaacccccta aaaacggcta acg

33

SEQ ID NO: 12

Length: 27

Type: DNA

Organism: Homo sapiens

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Sequence: 12

crccctaaaa tccccraaat crccgcg

27

SEQ ID NO: 13

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 13

accccracra ccrctacacc ccraacgtcg

30

SEQ ID NO: 14

Length: 31

Type: DNA

Organism: Homo sapiens

Sequence: 14

ctcttctaaa aaatcccrcr aactccggcc g

31

SEQ ID NO: 15

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 15

aaaacrcctt aaaatccccg aaatcgccg

29

SEQ ID NO: 16

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 16

aactccrcg gaccccaacc ccgacgaccg

30

SEQ ID NO: 17

5/17

Length: 23

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence: Oligonucleotide

which binds bisulfite-converted human GST-Pi gene

Sequence: 17

aaacctaaaaa aataaacaaa caa

23

SEQ ID NO: 18

Length: 23

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence: Oligonucleotide

which binds non-converted human GST-Pi gene

Sequence: 18

gggcctaggg agtaaacaga cag

23

SEQ ID NO: 19

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence: Oligonucleotide

which binds human GST-Pi gene

Sequence: 19

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cctttccctc tttcccarrt cccca

25

SEQ ID NO: 20

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence: Oligonucleotide

which binds bisulfite-converted human GST-Pi gene

Sequence: 20

tttggtattt ttttcgggt tttag

25

SEQ ID NO: 21

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence: Oligonucleotide

which binds non-converted human GST-Pi gene

Sequence: 21

cttggcatcc tcccccgggc tccag

25

SEQ ID NO: 22

Length: 26

Type: DNA

Organism: Artificial Sequence

Feature:

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Other Information: Description of Artificial  
Sequence: Oligonucleotide  
which binds human GST-Pi gene

Sequence: 22

ggyagggaag ggaggyaggg gytggg

26

SEQ ID NO: 23

Length: 31

Type: DNA

Organism: Homo sapiens

Sequence: 23

ttatgtataa aatttgtata ttttgtatat g

31

SEQ ID NO: 24

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 24

tgttagattat ttaaggtag gagtt

25

SEQ ID NO: 25

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 25

aaacctaaaa aataaacaaa caacaaaa

27

SEQ ID NO: 26

Length: 29

Type: DNA

Organism: Homo sapiens

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Sequence: 26

aaaaaacctt tcccttttc ccaaatccc

29

SEQ ID NO: 27

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 27

tttgtgttt gtttatTTTT taggttt

27

SEQ ID NO: 28

Length: 26

Type: DNA

Organism: Homo sapiens

Sequence: 28

gggatttggg aaagaggaa aggttt

26

SEQ ID NO: 29

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 29

actaaaaact ctaaacccca tccc

24

SEQ ID NO: 30

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 30

aacctaatac taccttaacc ccat

24

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SEQ ID NO: 31

Length: 33

Type: DNA

Organism: Homo sapiens

Sequence: 31

aatcctcttc ctactatcta tttactccct aaa

33

SEQ ID NO: 32

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 32

aaaacctaaa aaaaaaaaaaa aaacttccc

29

SEQ ID NO: 33

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 33

ttgggtttat gttgggagtt ttgagtttt

29

SEQ ID NO: 34

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 34

ttttgtgggg agttggggtt tgatgttgt

29

SEQ ID NO: 35

Length: 29

Type: DNA

Organism: Homo sapiens

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Sequence: 35

ggttagt gtttagtatg gggtaatt

29

SEQ ID NO: 36

Length: 20

Type: DNA

Organism: Homo sapiens

Sequence: 36

tagtattagg ttagggtttt

20

SEQ ID NO: 37

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 37

aactctaacc ctaatctacc aacaacata

29

SEQ ID NO: 38

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 38

caaaaaactt taaataaacc ctccctacca

29

SEQ ID NO: 39

Length: 32

Type: DNA

Organism: Homo sapiens

Sequence: 39

gttttggtt taggttgtt tttaggttt ag

32

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SEQ ID NO: 40

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 40

gtttttagta ttttgtgtgt ggttagttttt

30

SEQ ID NO: 41

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 41

ttaatataaaa taaaaaaaaaat atatttacaa

30

SEQ ID NO: 42

Length: 34

Type: DNA

Organism: Homo sapiens

Sequence: 42

caaccccaa tacccaaaccc taatacacaat actc

34

SEQ ID NO: 43

Length: 26

Type: DNA

Organism: Homo sapiens

Sequence: 43

ggtttttagtt tttgggtgtt tggatg

26

SEQ ID NO: 44

Length: 26

Type: DNA

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Organism: Homo sapiens

Sequence: 44

ttttttgg ttttagtatat gtgggg

26

SEQ ID NO: 45

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 45

ataactaaaaa aactatttc taatcctcta

30

SEQ ID NO: 46

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 46

ccaaactaaa aactccaaaa aaccactaa

29

SEQ ID NO: 47

Length: 38

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 47

tgtaaaaacga cggccagtgg gatttgggaa agaggaa

38

SEQ ID NO: 48

Length: 38

Type: DNA

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Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 48

tgtaaaacga cggccagttg ttgggagttt tgagtttt 38

SEQ ID NO: 49

Length: 31

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 49

tgtaaaacga cggccagtta gtatttagtt a 31

SEQ ID NO: 50

Length: 37

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 50

tgtaaaacga cggccagtgt tttgagtatt tgttgtg 37

SEQ ID NO: 51

Length: 35

Type: DNA

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Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human  
GST-Pi oligonucleotide

Sequence: 51

tgtaaaacga cggccagtgt ttttagtata tgtgg

35

SEQ ID NO: 52

Length: 499

Type: DNA

Organism: Homo sapiens

Sequence: 52

tgcagatcac ctaaggtcag gagttcgaga ccagccccgc caacatggtg aaaccccgtc 60  
tctactaaaa atacaaaaat cagccagatg tggcacgcac ctataattcc acctactcgg 120  
gaggctgaag cagaattgct tgaacccgag aggccggaggt tgcagtgagc cgccgagatc 180  
gcgccactgc actccagcct gggccacagc gtgagactac gtcataaaaat aaaataaaaat 240  
aacacaaaaat aaaataaaaat aaaataaaaat aataaaaataa aataaaaataa 300  
aataaaaataa aataaaaataa agcaatttcc tttcctctaa gcggcctcca cccctctccc 360  
ctgcccctgtg aagcgggtgt gcaagctccg ggatcgcagc ggtcttaggg aatttcccc 420  
cgcgatgtcc cggcgccca gttcgctgctg cacaacttcgc tgcggtcctc ttccctgctgt 480  
ctgtttactc cctaggcccc 499

SEQ ID NO: 53

Length: 316

Type: DNA

Organism: Homo sapiens

Sequence: 53

gggacctggg aaagagggaa aggcttcccc ggccagctgc gcggcgactc cggggactcc 60  
agggcgcccc tctgcggccg acgccccgggg tgcagcggcc gccggggctg gggccggcgg 120  
gagtcccgccg gaccctccag aagagcggcc ggcggcgtga ctcagcactg gggcggagcg 180  
gggcgggacc acccttataa ggctcggagg ccgcgaggcc ttcgctggag tttcgccgcc 240  
gcagtcttcg ccaccagtga gtacgcgcgg cccgcgtccc cggggatggg gctcagagct 300

15/17

cccagcatgg ggccaa

316

SEQ ID NO: 54

Length: 603

Type: DNA

Organism: Homo sapiens

Sequence: 54

cagcatcagg cccgggctcc cggcagggtc cctcgccccac ctcgagaccc gggacggggg 60  
cctaggggac ccaggacggtc cccagtgccg ttagcggctt tcagggggcc cggagcgcc 120  
cggggaggga tgggacccccg ggggcggggg gggggggcag gctgcgctca ccgcgcctt 180  
gcattcctccc ccgggctcca gcaaactttt ctttgttcgc tgcagtgcgg ccctacaccg 240  
tggtctatTT cccagttcga ggttaggagca tgtgtctggc agggaaaggga ggcaggggct 300  
ggggctgcag cccacagccc ctcgcccacc cggagagatc cgaacccct tatccctccg 360  
tcgtgtggct tttacccccc gcctccttcc tttccccgc ctctcccgcc atgcctgctc 420  
cccgccccag ttttgtgtga aattttcgga ggaacctgtt tacctgttcc ctccctgcac 480  
tcctgacccc tccccgggtt gctgcgaggc ggagtcggcc cggtccccac atctcgta 540  
tctccctccc cgcaggccgc tgcgccggccc tgcgcatgct gctggcagat cagggccaga 600  
gct

603

SEQ ID NO: 55

Length: 266

Type: DNA

Organism: Homo sapiens

Sequence: 55

gctctgagca cctgctgtgt ggcagtctct catccttcca cgcacatcct cttccctcc 60  
tcccaggctg gggctcacag acagccccct gttggccca tccccagtga ctgtgtgtt 120  
atcaggcgcc cagtcacgctg gcctgctccc ctccacccaa ccccagggtctatggaa 180  
gaccagcagg aggcagccct ggtggacatg gtgaatgacg gcgtggagga cctccgctgc 240  
aaatacatct ccctcatcta cacca

266

SEQ ID NO: 56

Length: 287

Type: DNA

Organism: Homo sapiens

16/17

Sequence: 56

tccccctgct ctcagcatat gtggggcgcc tcagtgcgg gcccaagctc aaggcattcc 60  
tggcctcccc ttagtacgtg aacctccccca tcaatggcaa cggaaacag tgagggttgg 120  
ggggactctg agcgggaggc agagtttgc ttccttc caggaccaat aaaatttcta 180  
agagagctac tatgagcact gtgttcctg ggacggggct taggggttct cagcctcgag 240  
gtcggtgaaa gggcagagca gaggactaga aaacagctcc tccagca 287

SEQ ID NO: 57

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 57

ataaaaataaa ataaaataaa ataaaagcaat ttcccttcct ctaagcgccc tccacccctc 60  
tccccctgccc tgtgaagcgg gtgtgcaagc tccgggatcg cagcggtctt aggaatttc 120  
cccccgcat gtccccggcgc gccagttcgc tgcgacact tcgctgcggc ccttttcctg 180  
ctgtctgttt actccctagg ccccgctggg gacctggaa agagggaaag gctccccgg 240  
ccagctgcgc ggcgactccg gggactccag ggcgcctc tgcggccgac gcccgggtg 300  
cagcggccgc cggggctggg gccggcgaaa gtccgcggg ccctccagaa gagcggccgg 360  
cgccgtgact cagcactggg gcggagcggg gcgggaccac ccttataagg ctggaggcc 420  
gcgaggcctt cgctggagtt tcggccgc agtcttcgac accagttagt acgcgcggcc 480  
cgcgtccccg gggatggggc tcagagctcc cagcatgggg ccaa 524

SEQ ID NO: 58

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 58

ataaaaataaa ataaaataaa ataaaagtaat ttttttttt ttaagtgggtt tttatttttt 60  
ttttttgttt tgtgaagtgg gtgtgttaagt tttgggattt tagtgggttt aggaattttt 120  
tttttgtat gttttgggtgt gttagttgt tttgttatatt ttgttgggtt tttttttttt 180  
ttgtttgttt attttttagg tttgttggg gatggggaa agagggaaag gtttttttgg 240  
ttagttgtgt ggtgattttgg gggattttag ggtgtttttt tttgtgtgtt gttgggggtg 300  
tagtgggtgt tgggggttggg gttgggtggg gtttggggaa ttttttagaa gagtgggtgg 360

17/17

tgttgtgatt tagtattggg gtggagtgaa gttggattat tttataagg tttggaggtt 420  
gtgaggaaaa ttgtggagtt ttgttgtt agttttgtt attagttagt atgtgtggtt 480  
tgtgttttgc gggatggggtt ttagagttt tagtatgggg tttaa 524

SEQ ID NO: 59

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 59

ataaaaataaa ataaaataaa ataaagtaat tttttttttt ttaagcgggtt tttatTTTTT 60  
ttttttgttt tgtgaagcgg gtgtgttaat ttccggatcg tagcggaaaa agggaaattttt 120  
ttttcgcgtat gtttcggcgc gtttagttcgt tgcgtatatt tcgttgcgggt ttttttttgc 180  
ttgtttgttt attttttagg tttcgttggg gatTTGGAA agaggaaag gttttttcgg 240  
tttagttgcgc ggcgatttcg gggatttttag ggcgtttttt tgcggtcgac gttcgggggtg 300  
tagcggtcgt cgggggttggg gtcggcggga gttcgcggga ttttttagaa gagcggtcgg 360  
cgtcgtgatt tagtattggg gcggagcggg gcgggattat tttataagg ttcggaggtc 420  
gcgaggtttt cggtggagtt tcgtcgtcgt agttttcggtt attagttagt acgcgcgggtt 480  
cgcgttttcg gggatggggtt ttagagttt tagtatgggg tttaa 524